Sensitivity of *Escherichia coli* to Viral Nucleic Acid, X

Ba^{2+}-Induced Competence for Transfecting DNA

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Effect of alkaline earth metal ions on induction of the competence for DNA transfection was investigated. Unlike spheroplasts, the bulk of the bacteria treated with these ions retains colony-forming ability. The order of effectiveness for transfection of ΦA replicative-form DNA has been found to be $Ba^{2+}>Ca^{2+}>Sr^{2+}>Mg^{2+}$. The competence of Ba^{2+}-treated cells is 3 to 5 times higher than that of Ca^{2+}-treated bacteria and about 40 times higher than that of lysozyme-EDTA spheroplasts. The Ba^{2+}-dependent transfection is cryophilic and formation of the infective complex occurs very rapidly at 0 °C, but not at 37 °C.

Introduction

In 1970, Mandel and Higa [1] reported that cells of *Escherichia coli* treated with chilled calcium chloride were readily transfected by lambdoid phage DNA. The calcium chloride method is very simple and mild as compared with spheroplast- or helper phage-systems. Moreover, bulk of the cells remains viable after the Ca^{2+} treatment. Taking advantage of these properties of the Ca^{2+} system, not only transfection but also transformation by various DNA species has been achieved in *E. coli* [2–6]. In order to elucidate the DNA-uptake mechanism as well as to clarify various factors and conditions affecting the practical use, we have investigated the idiosyncrasy of the Ca^{2+}-dependent transfection system in detail [7, 8]. In an extension of these studies, we have compared the competence-inducing effect of other alkaline earth metal ions with that of Ca^{2+}. Present results show that the most effective ion for the competence development is Ba^{2+}.

Materials and Methods

Unless otherwise specified, *E. coli* strain C was used throughout. The SS DNA and the RF of ΦA were prepared as described previously [9]. The infectivity of these DNA was calibrated by a lysozyme-EDTA spheroplast system [2, 10]. For induction of the competence, bacteria were grown in nutrient broth at 37 °C with shaking. At a density of $A_{660}=0.7$ (measured by a Bausch & Lomb Spectronic 20 spectrophotometer), the culture was chilled in ice-water, sedimented, and suspended in 1/2 volume of chilled 0.1 M $BaCl_2$. After standing at 0 °C for 30 min the bacteria were collected by a centrifugation, resuspended in chilled 0.1 M $BaCl_2$ at a density of $A_{660}=15$ and preserved at 0 °C. Transfection was carried out as follows: To the competent cell suspension (usually 0.1 ml), 1/2 volume of DNA in chilled 50 mM Tris-HCl, pH 7.5 was added and mixed at 0 °C. After chilling for 20 min, the infected complex was diluted with chilled 0.1 M $BaCl_2$ and plated with the indicator bacteria on nutrient agar. Treatment with other alkaline earth metal ion was performed similarly.

Results

In preliminary experiments, cells of *E. coli* C were treated with chilled 0.05 M solutions of $CaCl_2$, $BaCl_2$ or $SrCl_2$ and their competence for ΦA RF was compared. The relative efficiency of competence induction was found to be $Ba^{2+}:Ca^{2+}:Sr^{2+}=1:0.24:0.10$. Consequently, several conditions for the Ba^{2+}-dependent transfection were further examined. As shown in Fig. 1, concentration of $BaCl_2$ required to induce maximal competence for RF was nearly 0.1 M and that for SS was about 0.08 M. Competence of the bacteria treated with 0.1 M solutions of alkaline earth metal compounds is presented in Table I. Efficiency of SS transfection was

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Abbreviations: SS, single-stranded virus DNA; RF, double-stranded replicative form DNA.

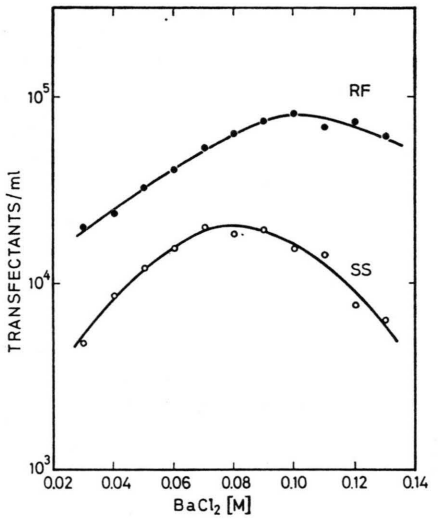

Fig. 1. Effect of $BaCl_2$ concentration on induction of the cellular competence. Bacteria were treated with various concentrations of chilled $BaCl_2$ and the competence for ΦA RF (●) or SS (○) was determined.

relatively high in $CaCl_2$-treated cells and considerably low in $SrCl_2$-treated bacteria. In production of competence for RF, however, $BaCl_2$ was 3 to 5 times efficient than $CaCl_2$ and the maximal competence of the Ba^{2+}-treated cells was about 40 times higher

Table I. Competence of the cells treated with alkaline earth metal ions. Cells of *E. coli* C treated with each compound (0.1 M) were tested for their competence for ΦA SS and RF. Figures in parentheses indicate ratio of the cellular competence to that of the bacteria treated with 0.1 M $CaCl_2$.

DNA	Plaque yield				
	$MgCl_2$	$CaCl_2$	$SrCl_2$	$BaCl_2$	$Ba(NO_3)_2$
SS	2.6×10^6 (0.87)	3.0×10^6 (1)	3.0×10^5 (0.10)	2.0×10^6 (0.66)	1.4×10^6 (0.45)
RF	1.3×10^6 (0.026)	5.1×10^7 (1)	2.6×10^7 (0.51)	2.1×10^8 (4.1)	1.2×10^8 (2.4)

than that of lysozyme-EDTA spheroplasts. It is clear that Ba^{2+} ion *per se* is the principal factor in competence induction, since $Ba(NO_3)_2$ as well is highly effective. As reported previously [8] Mg^{2+}, even at 0.1 M, was rather ineffective for RF transfection.

Colony-forming ability of the bacteria was not significantly affected by the treatment with alkaline earth metal ions (Table II). In chilled 0.1 M $BaCl_2$, the cellular competence could be stably maintained for 2 to 3 days. Fig. 2 shows a relationship between DNA concentration and the yield of transfectants. Over a wide range, the number of infective centers

Table II. Viability of cells suspended in alkaline earth metal salt solution. Bacteria were grown in nutrient broth at 37 °C with shaking and harvested at a density of A_{660} = 0.7. Ten ml aliquots of the chilled bacteria (1.5×10^9 viable cells/ml) were sedimented and suspended in 5 ml of the indicated media chilled at 0 °C. Viability of the treated cells was assayed 30 min or 24 h after suspension.

Media	1st suspension (after 30 min)	2nd suspension (after 30 min)	2nd suspension (after 24 h)
0.1 M $BaCl_2$	1.4×10^{10}	1.3×10^{10}	1.2×10^{10}
0.1 M $Ba(NO_3)_2$	1.4×10^{10}	1.2×10^{10}	1.1×10^{10}
0.1 M $CaCl_2$	1.5×10^{10}	1.3×10^{10}	1.2×10^{10}
0.1 M $SrCl_2$	1.5×10^{10}	1.3×10^{10}	1.1×10^{10}

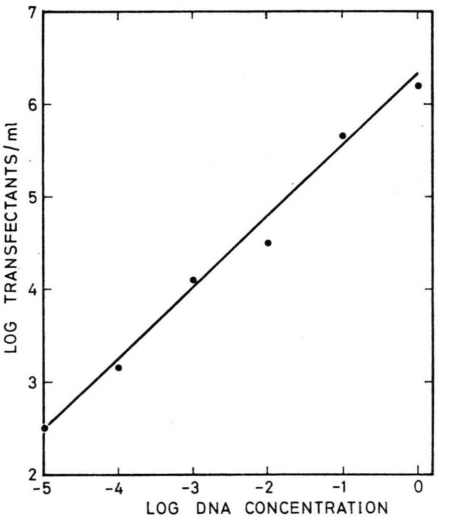

Fig. 2. Effect of DNA concentration. ΦA RF was serially diluted with 50 mM Tris-Cl, pH 7.5 and infectivity of each sample was assayed using the 0.1 M $BaCl_2$-treated hosts.

was directly proportional to the concentration of input DNA. In chilled $BaCl_2$ solution, formation of infective complex occurred very rapidly and within 20 to 30 sec after mixing, the yield of infective centers reached the plateau level (Fig. 3). The complex formed at 0 °C was stable in chilled $BaCl_2$ solution, but the infectivity was reduced by simple dilution into nutrient broth. Upon brief incubation at 37 °C, the complex became resistant to the interference by nutrient broth, though the heat pulse *per se* reduced yield of the transfectants by about 50%. (In practical use therefore, the infected complex has to be diluted with chilled $BaCl_2$ solution, without heat pulse.) As in Ca^{2+}-dependent transfection, low temperature condition is essential at least for an early phase of Ba^{2+}-dependent DNA-uptake. Thus, when DNA and the recipient cell suspension were mixed at 37 °C for 2 min and plated directly,

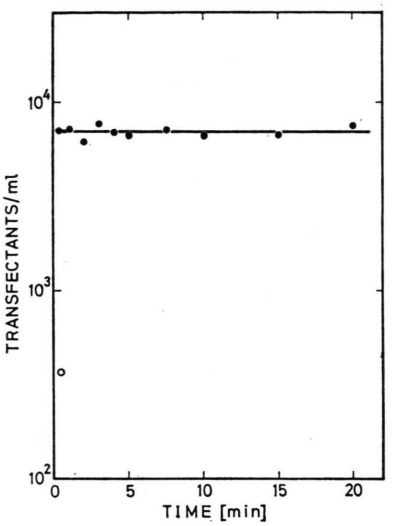

Fig. 3. Time course of infection at 0 °C. The competent cell suspension in 0.1 M $BaCl_2$ was mixed at 0 °C with ΦA RF. At the indicated time, aliquot was removed and plated directly (●) or after dilution with nutrient broth (○).

plaque yield was less than 5% of the control mixed at 37 °C and then cooled for 2 min at 0 °C before plating.

Discussion

Present data demonstrate that Ba^{2+} is more effective than Ca^{2+} in induction of the competence for transfection of ΦA RF. The Ba^{2+}-treated *E. coli* was efficiently infected by ΦX 174 RF or double-stranded ΦT DNA as well (unpublished observation). In current experiments on DNA transformation in *E. coli*, the Ca^{2+}-treated cells are generally used as the recipients. The transformation in *E. coli* is, however, less efficient and requires larger amounts of DNA as compared with transformation in other bacteria. Since the Ba^{2+}-treated cells are mostly viable, this method may potentially be useful for transformation in *E. coli* or allied bacilli.

The Ba^{2+}-dependent transfection system is similar to the Ca^{2+}-dependent system in many properties. The Ca^{2+}-dependent transfection is strangely cryophilic and this idiosyncrasy has led us to hypothesize that early phase of the DNA-uptake depends on crystallization of surface (phospho)lipids [8]. Although Ba^{2+}-induced competence is consistent with the hypothesis, further work is needed to elucidate the complicated mechanism of DNA penetration through cell envelope.

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